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AROMATIC OILS FROM SASKATCHEWAN GROWN CROPS AS NOVEL REDUCED RISK PEST CONTROL PRODUCTS

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AROMATIC OILS FROM SASKATCHEWAN-GROWN CROPS AS NOVEL, REDUCED-RISK PEST CONTROL PRODUCTS

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1. ABSTRACT

The potential to use aromatic oils from Saskatchewan-grown crops as novel, reduced-risk pest control products wwere assessed in a series of trials. The growth of pure cultures of six pathogens, originally isolated from Saskatchewan crops, was assessed in the presence and absence of aromatic oils. Aromatic oils prepared from anise, caraway, coriander and dill crops, along with commercially available purified components from aromatic oils (carvone and allyl isothiocyanate (AITC) were tested. All the aromatic oils and the purified components treatments were effective in reducing growth of the pathogens in pure culture. The efficacy of the aromatic oils was dosage dependant and there appeared to be some degree of specificity – in that certain oils were more effective against certain pathogens. The effect of treatment with low concentrations of aromatic oils on control of seed-borne diseases was assessed using several crop species. There was no reduction in the levels of *Fusarium* species on barley, *Ascochyta lentis* on lentil, *A. pinodes* on pea or *A. rabiei* on chickpea at the concentrations tested. Aromatic oil treatments also failed to reduce storage rot of strawberry over a three day period. *Rhizoctonia solani* viability on potato skin appeared to be reduced, particularly by caraway, dill and AITC treatment but bacterial development was enhanced. Future tests on control of potato diseases should assess the impact of essential oil treatments on soft rot development during potato storage, as well as the impact of these treatments on sprout development.

2. EXECUTIVE SUMMARY

Plant diseases cause huge crop losses in Saskatchewan and worldwide, by damaging yields and reducing quality. Diseases that strike after harvest are particularly problematic as the proximity to time of consumption limits the range of pest control products that can be safely applied. There is an ongoing need to identify new, effective disease management products that are safe for the applicator, the consumer and the environment.

Saskatchewan farmers are able to produce a range of spice and herb crops that yield aromatic essential oils. These essential oils are already widely used in a range of foods and cosmetics, and have a well established record of environmental and consumer safety. Essential oils may also represent effective biofumigants for the control of diseases that develop after harvest and/or diseases that are spread by infested seed.

Development of pest control products based on Saskatchewan-grown aromatic oils would represent a market for additional acres of high value crops, would provide opportunites for value-added processing of the crops into their essential oil components, while also addressing the need to continue to develop environmentally friendly, economically sustainable methods of pest management.

In this study, essential oils extracted from locally grown speciality crops (anise, caraway, coriander, and dill) were evaluated as potential biocontrol agents for a range of post harvest and seed-borne diseases utilizing a simplified assay system. The effect of different concentrations of the oils (in the vapour phase) on the growth of pure cultures of a range of pathogens that damage field and specialty crops, both in the field and after harvest, was assessed. Purified carvone which is the dominant component in dill and coriander seed oil and allyl isothiocyanate (AITC) which is the dominant component in mustard oil were also evaluated.

The fungal pathogens were grown on agar plates while being exposed to the volatile oils, and the effect of the oils was determined by comparing the growth rates of the fungi in the presence and absence of the oils. Five tests were conducted, using different concentrations of the oils and different incubation temperatures.

The aromatic oils decreased growth of the pathogens in pure culture in a dosage dependent manner. Certain oils show potential for controlling specific pathogens at low concentrations, under temperature conditions typically encountered during field production and storage. These oils and treatment concentrations represented the focus of subsequent testing. Anise oil appears to have more specificity that the other oils.

The effect of treatment with aromatic oils on levels of seed-borne disease was evaluated using several crop species. There was no reduction in the levels of *Fusarium* species on barley, *Ascochyta lentis* on lentil, *A. pinodes* on pea and *A. rabiei* on chickpea at the concentrations of aromatic oils tested. The oil treatments had no impact on seed germination which suggests that the treatments were not phytotoxic. At the concentrations tested, the aromatic oil treatments also failed to reduce storage rot of strawberry over a three day period. However the viability of *Rhizoctonia solani* on potato skin appeared to be reduced, particularly by caraway, dill and AITC treatments but bacterial development was enhanced by these treatments. Future tests should assess the impact of any oil treatment on development of soft rot during potato storage, as well as the effect of the oils on sprout development of the tubers.

The efficacy of these oils for treating infected tissues will depend on the sensitivity of the pathogen when in or on the host, and on the effect of the oil on crop quality. The minimum dose of oil required to control infection of a host, the most effective oil for a particular disease, and the impact of treatment on quality of food product are all factors that need to be examined further.

3. TECHNICAL REPORT

3.1 Background

Plant diseases cause huge crop losses in Saskatchewan and worldwide, by damaging both yields and quality. Diseases that strike after harvest are particularly problematic as the proximity to time of consumption limits the range of pest control products that can be safely applied. There is an ongoing need to identify new, effective disease management products that are safe for the applicator, the consumer and the environment.

Saskatchewan has the potential to produce a range of crops (dill, mint, caraway, coriander, etc.) that yield aromatic essential oils. These oils are already widely used in a range of foods and cosmetics and have a well established record of environmental and consumer safety. There is very strong demand for new, safe, environmentally sound and ideally "organic" pest control products. Preliminary research conducted at the University of Saskatchewan and elsewhere suggests that the essential oils produced by Saskatchewan-grown crops may represent effective biofumigants for the control of diseases that develop after harvest and/or diseases that are spread by infested seed.

Development of pest control products based on Saskatchewan-grown aromatic oils would represent a market for additional acres of high value crops, would provide opportunites for value-added processing of the crops into their essential oil components, while also addressing the need to continue to develop environmentally friendly, economically sustainable methods of pest management.

The objectives of this project were to evaluate the essential oils extracted from locally grown speciality crops like anise, caraway, coriander and dill, as potential biocontrol agents for a range of post harvest and seed-borne diseases utilizing a simplified assay system. Oils that demonstrate pesticidal activity could be used as as a tool in the management of diseases of crops grown in Saskatchewan and elsewhere.

The most promising compounds identified in the initial screening trials were to be tested as potential biofumigants for control of diseases in enclosed environments (seed and product storages). The efficacy, optimum dosage and method of application, duration of efficacy and any issues with phytotoxicity were to be evaluated. The impact of the various treatments on quality and value of the treated commodity were also to be evaluated. It is important to determine whether the treatment enhances quality by reducing disease levels without compromising other aspects of quality, ie. appearance or flavor.

The design of product development initiatives and/or additional studies were to be based on the results of this project. If specific components or methods of application were found to be particularly effective, there may be the potential for patentable I.P.

In the preliminary laboratory trials, the disease control potential of the aromatic oils was evaluated using pure cultures of a range of pathogens that damage field and specialty crops, both in the field and after harvest. The impact of differing concentrations of the various essential oils on growth of these pathogens was evaluated. The oils were delivered in the vapor phase, as this represented the most efficient and effective means of application.

The chemical composition of essential oil extracts varies with the plant species, the tissue being extracted, the extraction method and the method of analysis. However, the composition of the essential oils tested in this project can be summarized as follows:

Source Plant	Primary oil constituents	Secondary compounds
Anise	trans-anethole	methyl chavicol
Caraway	carvone and limonene	
Coriander	linalool and limonene	
Dill	carvone and limonene	phellandrene
Mustard	Allyl-isothiocyanate	

Although the primary components may consitute over 90% of the essential oil, the less common secondary consituents may still be important in determining bioactivity and toxicity of the essential oil. For example, the primary constituents of the essential oils extracted from caraway and dill are similar (carvone and limonene), yet these oils smell quite different. This reflects the potential importance of the relatively less common secondary components.

3.2 Evaluation of aromatic oils as biocides

3.2.1 Evaluation of the effect of aromatic oils on the growth rate of fungal pathogens

Materials and Methods:

The biocidal potential of the aromatic oils and purified oil components was initially assessed in 2008 by determining their effect on the growth of a number of plant pathogens. The fungal pathogens were grown on agar plates while being exposed to the volatile oils, and the effect of the oils was determined by comparing the growth rates of the fungi in the presence and absence of the oils. Essential oils were extracted from locally grown anise, caraway, coriander and dill using an industrial scale distillation unit (P. LeDuc - Prairie Agriculture Machinery Institute). The oils were all steam extracted at 100°C and condensed in the 70°C temperature range. Two purified components of common aromatic oils were also tested : Carvone (R(-) Carvone, 98%, Sigma-Aldrich) and Allyl isothiocyanate (AITC, 98%, Fluka). Carvone is the primary component in the

essential oil extracted from caraway and dill. Carvone has been shown to limit the growth of a range of post-harvest pathogens (J. Velichka, 2005. The efficacy of R-(-)-carvone for control of fungal pathogens *in-vitro* and in postharvest storage of carrot and potato. Student thesis, University of Saskatchewan.) and a carvone-based product (Talent) is presently registered for post-harvest disease control in potatoes. AITC can be produced from Brassica species grown in Saskatchewan, and it has been shown to reduce potato dry rot in a previous study (M. Reaney, 2007. Industrial bioprocessing of high glucosinolate cruciferous plants - ADF# 20040548).

It is noteworthy both purified extracts pose increased risks for handling and application than the basic aromatic oils. Purified carvone is a strong solvent and skin irritant, while AITC is irritating to mucous membranes, the eyes and lungs.

The impact of the oils on the rate of mycelial growth of a number of plant pathogens was evaluated in five laboratory assays. The pathogens used in these initial assays were isolated from Saskatchewan crops (Table 3.2.1.1).

Table 3.2.1.1. Pathogens (and their origins) used to assess the biocidal potential of aromatic oils.

Pathogen	Disease caused	Host from which isolated
Rhizoctonia solani	Black scurf	potato
Botrytis sp.	Neck rot	onion
Sclerotinia sclerotiorum	White mould	cabbage
Colletotrichum coccodes	Black dot	potato
Fusarium sambucinum	Dry rot	potato
Fusarium graminearum	Head blight	Cereal seed

The six fungal pathogens were grown on potato dextrose agar (PDA) for a week to ten days to obtain actively growing cultures. Five mm plugs were cut from the edge of these colonies with a sterile cork borer and the plugs were placed in the centre of new PDA plates. The inoculated plates were allowed to grow at room temperature (20 - 22°C) for 24 hours to establish mycelial growth. The plates were then inverted and a 5.5 cm filter paper disc (Whatman #4) was placed on the inside of the plate lid. A 0.5 mL aliquot of the selected essential oil was placed on the filter disc and the plate was then sealed with parafilm. Three plates of each pathogen were treated with the same oil and all the plates treated with the same oil were stored in a plastic container with a tight lid, sealed with parafilm to prevent fumes dipersing into the laboratory (Figure 3.2.1.1).

The plates in the control (no chemical added) container were examined periodically and once the culture had grown to the edge of the control plates, the diameter of the mycelial

growth in the treated plates was measured for that pathogen. The maximum growth measured 8.2cm, at which point the petri plate was completely covered. The mimimum growth was 0.5cm, when there was no growth from the initial 5mm plug. The fungal cultures grew at different rates, with *R. solani* being the fastest growing, covering the control plate in 3-4 days, and *C. coccodes* being the slowest (12-13 days).

The diameter of the colony was measured twice (the two measurements being at right angles to each other) and the average diameter was recorded for each plate.

Five trials were conducted, using different concentrations of the essential oils, and different incubation temperatures. Initially dimethyl-sulphoxide (DMSO) was used as a diluant for the oils but mineral oil (light white oil, Sigma) was used for the subsequent tests as it appeared as effective as DMSO, while bring less hazardous to use. The essential oil concentrations and conditions for the five test runs were as follows:

Test 1
Anise, caraway, coriander and dill - oil as supplied
AITC – 4% in DMSO
Carvone – 4% in DMSO
DMSO check
All pathogens at room temperature.

Test 2
Anise, caraway, coriander and dill – diluted to 50% aromatic oil in mineral oil
AITC – 4% in mineral oil
Carvone – 4% in mineral oil
Mineral oil check
All pathogens at room temperature.

Test 3
Same essential oil concentrations as test 2.
Three pathogens used – *R. solani*, *S. sclerotiorum*, *F. sambucinum*. These pathogens were tested at a lower temperature as they typically cause problems under cooler storage conditions.
Plates incubated at 15°C.

Test 4 and 5
Anise, caraway, coriander and dill – diluted to 5% aromatic oil in mineral oil
AITC – 0.5% in mineral oil
Carvone – 0.5% in mineral oil
Mineral oil check
All pathogens at room temperature.

Results and Discussion:

In the first test, when DMSO was used as a dilutant, the rate of growth of *S. sclerotiorum* (Ss) and *F. graminearum* (Fg) were not affected by the presence of the DMSO but the other pathogens showed a slight reduction in growth due to the DMSO (Table 3.2.1.2).

The growth of *R. solani* (Rs), Ss, *C. coccodes* (Cc) and *F.sambucinum* (Fs) was reduced signifiantly by all the essential oil treatments, with no significant differences between the different oils. There were significant differences in the effect of the oils on the growth of *Botrytis* sp. (B) and Fg. Although all oils greatly reduced the growth in comparison with the untreated plates, it appeared that the growth of B was most effectively suppressed by the caraway oil treatment. The growth of Fg was most reduced by the dill, coriander and caraway oil treatments.

In test two, mineral oil was used as the dilutant, and the four spice oils were diluted to 50% strength, while carvone and AITC were kept at the 4% dilution used in test one. The mineral oil appeared to reduce growth relative to the untreated check for Ss and Cc but did not affect the other fungi (Table 3.2.1.3). Carvone had significantly less effect than the other oils for several of the fungi. The four essential oils were as effective at 50% strength as when used at full-strength (see test no. 1). The 4% AITC was again as effective in reducing mycelial growth as the essential oils.

Table 3.2.1.2. The effect of aromatic oils and DMSO on the mycelial growth of six plant pathogens.

Aromatic oil treatment	**Mycelial growth of fungal colonies of pathogens (maximum 8.2cm, minimum 0.5cm):**					
	R. solani	***Botrytis* sp.**	***S. sclerotiorum***	***C. coccodes***	***F. sambucinum***	***F. graminearum***
Coriander (100%)	1.43c*	1.13dc	0.68b	0.70c	1.22c	0.75cd
Caraway (100%)	1.40c	0.80e	0.65b	0.75c	0.98c	0.78cd
Anise (100%)	1.55c	1.03de	0.67b	0.83c	1.13c	0.85bc
Dill (100%)	1.40c	0.80d	0.67b	0.70c	1.05c	0.72d
AITC (4%)	1.50c	1.00de	0.75b	0.68c	1.03c	0.95b
Carvone (4%)	1.32c	1.32c	0.65b	0.82c	1.10c	0.90b
DMSO	7.72b	7.92b	8.00a	6.13b	7.47b	8.20a
Control	8.20a	8.20a	8.20a	8.20a	8.20a	8.20a
Coefficient of variance	*7.03*	*5.28*	*5.54*	*12.50*	*8.94*	*2.35*

* values followed by the same letter are not significantly different, using LSD (α=.05)

Three of the fungi were tested at 15°C because they typically cause damage at lower temperatures, either in the ground during cool, spring weather (Rs) or in cold storage (Fs and Ss). Anise oil appeared particularly effective in reducing the growth of Rs at both room and cool temperatures. The AITC was less effective at the cooler temperature (Table 3.2.1.4). All treatments significantly reduced growth of Ss at both room and cool temperatures. Carvone and anise oils were less effective in reducing the growth of Fs at both room and cool temperature. It is interesting that the anise oil had a different effect on Rs and Fs, indicating that some oils may be more suitable for the control of certain pathogens.

Table 3.2.1.3. The effect of aromatic oils and mineral oil on the mycelial growth of six plant pathogens at room temperature.

Aromatic oil treatment	**Mycelial growth of fungal colonies of pathogens (maximum 8.2cm, minimum 0.5cm):**					
	R. solani	***Botrytis* sp.**	***S. sclerotiorum***	***C. coccodes***	***F. sambucinum***	***F. graminearum***
Coriander (50%)	1.42c*	0.85c	0.5c	0.58d	0.95c	0.97c
Caraway (50%)	1.45c	0.98c	0.5c	0.60d	1.07c	0.70c
Anise (50%)	1.2d	0.90c	0.5c	0.53d	2.25b	1.00c
Dill (50%)	1.45c	1.07c	0.5c	0.57d	0.95c	0.90c
AITC (4%)	0.90e	0.73c	0.62c	0.58d	0.98c	1.15c
Carvone (4%)	1.67b	2.18b	0.63c	2.63c	2.78b	1.80b
Mineral oil	8.2a	7.55a	6.3b	5.27b	8.20a	8.20a
Control	8.2a	8.2a	8.2a	8.20a	8.20a	8.20a
Coefficient of variance	*4.12*	*14.77*	*23.57*	*15.40*	*15.29*	*11.57*

* values followed by the same letter are not significantly different, using LSD (α=.05)

In test four and five the concentrations of all the oils were reduced to 1:20 of full strength. The growth of Rs was reduced by all the oils in both test four and five, although the carvone treatment was not as effective as the other treatments (Tables 3.2.1.5 and 3.2.1.6). Carvone was not effective in reducing the growth of B, while AITC was the most effective treatment on this pathogen in both tests. Anise oil also appeared less effective in controlling this fungus. Diluted carvone and anise were also not effective in controlling Ss. AITC and anise had the greatest effect on Cc, while caraway oil and AITC

had the greatest effect on both Fusarium species. Dilute dill oil was also effective in reducing growth of Fg and Fs.

Table 3.2.1.4. The effect of aromatic oils and mineral oil on the mycelial growth of three plant pathogens at 15°C.

Aromatic oil treatment	Mycelial growth of fungal colonies of pathogens (maximum 8.2cm, minimum 0.5cm):		
	R. solani	*S. sclerotiorum*	*F. sambucinum*
Coriander (50%)	1.55bc*	0.60c	1.13cd
Caraway (50%)	1.65b	0.57c	0.93d
Anise (50%)	1.33c	0.82c	2.33bc
Dill (50%)	1.53bc	0.70c	0.83d
AITC (4%)	1.47bc	0.60c	0.93d
Carvone (4%)	1.68b	0.50c	3.58b
Mineral oil	8.20a	6.98b	7.63a
Control	8.20a	7.63a	8.13a
Coefficient of variance	*3.94*	*15.78*	*22.95*

* values followed by the same letter are not significantly different, using LSD (α=.05)

Table 3.2.1.5. The effect of aromatic oils and mineral oil (at different concentrations) on the mycelial growth of six plant pathogens at room temperature.

Aromatic oil treatment	Mycelial growth of fungal colonies of pathogens (maximum 8.2cm, minimum 0.5cm):					
	R. solani	*Botrytis* sp.	*S. sclerotiorum*	*C. coccodes*	*F. sambucinum*	*F. graminearum*
Coriander (5%)	1.07de*	3.63c	1.47b	6.08c	6.97b	4.15c
Caraway (5%)	1.03de	1.57de	0.55c	2.13d	2.60d	2.23de
Anise (5%)	1.47c	6.98b	8.20a	0.73e	8.20a	3.08cd
Dill (5%)	1.18d	2.0d	0.78c	2.48d	2.97c	1.40e

AITC (0.5%)	0.87e	0.68e	0.80c	0.50e	1.00e	1.13e
Carvone (0.5%)	7.60b	8.20a	8.02a	7.22b	6.82b	3.90c
Mineral oil	8.20a	7.72ab	8.07a	7.95ab	8.20a	6.62b
Control	8.20a	8.20a	8.02a	8.20a	8.20a	8.20a
Coefficient of variance	*4.10*	*11.86*	*4.27*	*9.62*	*3.33*	*16.67*

* values followed by the same letter are not significantly different, using LSD (α=.05)

Tests four and five show that the results are consistent and that some fungi are more responsive to certain oils. The oils also appear to be effective at very low concentrations in this type of laboratory test.

Table 3.2.1.6. The effect of aromatic oils and mineral oil (at different concentrations) on the mycelial growth of six plant pathogens at room temperature (Test 4 repeated).

Aromatic oil treatment	**Mycelial growth of fungal colonies of pathogens (maximum 8.2cm, minimum 0.5cm):**					
	R. solani	***Botrytis* sp.**	***S. sclerotiorum***	***C. coccodes***	***F. sambucinum***	***F. graminearum***
Coriander (5%)	1.23d*	3.65c	1.17c	4.95b	5.93b	2.20cd
Caraway (5%)	1.28cd	1.40d	0.50d	2.32d	2.87cd	1.10ef
Anise (5%)	1.65c	5.87b	7.83a	0.87e	8.07a	2.13cde
Dill (5%)	1.32cd	1.93cd	0.62d	3.18c	3.23c	1.27def
AITC (0.5%)	1.22d	0.50d	0.50d	0.52e	1.12d	0.70f
Carvone (0.5%)	6.97b	8.20a	7.20b	5.22b	7.33ab	2.88c
Mineral oil	8.20a	8.20a	8.10a	7.73a	8.20a	6.87b
Control	8.20a	8.20a	8.02a	8.13a	8.20a	8.10a
Coefficient of variance	*6.07*	*20.85*	*5.78*	*11.67*	*18.76*	*18.95*

* values followed by the same letter are not significantly different, using LSD (α=.05)

Conclusions:

The laboratory tests indicated that the aromatic oils and purified oil components showed potential for controlling specific pathogens under temperature conditions typically encountered during field production and storage. As expected, efficacy was dosage dependant but a significant degree of pathogen control was possible with fairly dilute treatments. Efficacy at low dosages is obviously desirable from the perspective of cost efficiency. It was interesting to note that anise appeared to be more pathogen specific that the other oils.

3.2.2. Evaluation of the effect of aromatic oils on the survival of fungal pathogens in host tissue

The efficacy of the aromatic oils for treating infected host tissues was evaluated using infected seed as a model system. Tests were performed on infected seed from a number of hosts. The potential to use the aromatic oils to control pathogens on strawberry fruit and potato tubers was also assessed.

a) SEEDS

Materials and Methods:

The seeds used were:

Chickpea (CDC Chico) infected with *Ascochyta rabiei*, harvested from a fungicide trial at University of Saskatchewan in 2008
Pea (Eclipse), 22.5% of sample infected with *Ascochyta pinodes*, obtained from Discovery Seed Lab
Lentil (Grandora), 57.3% infected with *Ascochyta lentis*, obtained from Discovery Seed Lab
Barley (CDC Trey), 41.5% infected with *Fusarium* species, 8.5% infected with *F. graminearum*, obtained from Discovery Seed Lab.

The oil treatments used were the same as those used in the plate tests reported in the previous section, except that the purified carvone was not included as it was not as consistently effective as the other oils in the plate tests:

Based on the results from the plate assay, the essential oils from anise, caraway, coriander and dill were diluted to 5% aromatic oil using mineral oil. The AITC was diluted to 0.5% in mineral oil. A mineral oil check was included in the design along with a no-treated check.

Seeds were counted out at random from the samples and 40 seeds of each plant type were placed in white, loosely woven, nylon fabric 12 cm squares that were secured with a twist-tie to form little packets of seed. The total sample of infected peas was small and only 19 seeds per packet were placed in the treatment containers for this crop.
The seed packets were placed on a wire mesh platform inserted into the same plastic containers used in the initial testing of the oils (Figure 3.2.1.1.). The platform was approximately 10 cm above the bottom of the container where a glass dish containing a filter paper disc (Whatman #4) had been placed. One ml of the treatment oil was pipetted onto the filter paper and the lid was placed tightly on the plastic tub, which was also sealed with parafilm. Each tub contained a packet of the four seed types, and there were three replicate tubs for each treatment.

The tubs were incubated at room temperature for two weeks and were then opened and all seed packets were removed. Each set of seeds was then surface sterilized in 50% household bleach for two minutes, rinsed in 10% bleach twice, and allowed to dry on sterile filter paper in the laminar flow hood. This technique was employed to remove surface contaminants from the seed, to allow assessment of the impact of the oil treatments on the seed-borne pathogens.

The surface sterilized seeds were plated onto potato dextrose agar (PDA) contained in sterile plastic petri dishes (Figure 3.2.2.1). Eight seeds were evenly spaced in each dish, and the dishes were incubated at room temperature and examined for fungal growth after 5-7 days.

Results and Discussion:

The growth of *Fusarium* species on the barley seed was evaluated after five days (Figure 3.2.2.2) and the pathogen levels on the other seeds were evaluated after seven days. All the seeds had begun to germinate and there were no signs that the presence of the oils had inhibited germination.

In the barley seed samples fungal colonies were growing out of all the seeds, and the colonies could be identified as mainly *Fusarium*, *Cochliobolus*, *Alternaria* or *Cladosporium* species (Figure 3.2.2.3). *Cochliobolus* and *Fusarium* are seed-borne pathogens of barley, while *Alternaria* and *Cladosporium* are generally considered to be saprophytic or weak pathogens. The average percentages of seed infected with *Fusarium* species (mainly *F. graminearum*) are shown in Table 3.2.2.1. The highest level of infection was present when there was no treatment with oil, but there was no significant difference between any of the treatments. The reduction in disease level shown by the caraway oil is due to a low value in one replicate, and this result is unlikely to be a real effect, given that the other two replicates would produce an average similar to the averages of the other oils.

Table 3.2.2.1. Effect of aromatic oil treatment on the percentage of seed infected with specific fungal pathogens, after incubation on PDA plates at room temperature.

Aromatic oil treatment	Average % of seeds infected (n=120) - Seed type/Pathogen			
	Barley/*Fusarium* species	Lentil/*A. lentis*	Pea/*A. pinodes* (n=57)	Chickpea/*A. rabiei*
Coriander (5%)	25.8	56.7	0	20.8
Caraway (5%)	17.5	47.5	3.5	19.2
Anise (5%)	30.0	55.8	3.5	20.0
Dill (5%)	23.3	41.2	7.0	17.5
AITC (0.5%)	23.3	54.2	5.3	17.5
Mineral oil	20.8	45.0	1.8	19.2
Control	27.5	55.0	3.5	14.2
Coefficient of variance	*30.5*	*13.5*	*114.0*	*34.4*

The overall average percentage of *Fusarium* infected seed determined in this test is 10-20% lower than determined at the Discovery Seed Lab, and this may be due to the seed having been stored for up to a year since the original test was carried out.

The percentage of lentil seed infected with *Ascochyta lentis* was not significantly affected by the aromatic oil treatments (Table 3.2.2.1) and the level of seed infection was only slightly lower than that determined by the Discovery Seed Lab. The majority of the seeds had started to germinate on the plates and there was no difference in the amount of germination between the oil treatments.

The percentage of peas infected with *Ascochyta* was appreciably lower than recorded by Discovery Seed Lab, but only a small number of seeds were tested in the oil treatments because of the small size of the original sample. The high coefficient of variance value reflects the low levels of disease present in this test. The coriander treatment reduced the *Ascochyta* isolates to zero, but mineral oil alone also reduced the number of isolates that grew out of these seeds (Table 3.2.2.1).

The percentage of chickpea seeds contaminated with *Ascochyta rabiei* was not significantly affected by the presence of any of the oils (Table 3.2.2.1).

Conclusions:

The lack of control of the seed pathogens by the aromatic oils was disappointing, given the observed reduction in fungal growth seen when the essential oils were used in the agar plate assays. It should be noted that this trial utilized relatively dilute (1:20) solutions of essential oils. As none of the treatments appeared to have any negative impact on seed germination it is possible that higher concentrations of oils could be used without undue risk to crop development.

b) FRUIT

Materials and Methods:

Botrytis grey mould is a common cause of post-harvest loss in strawberry. As the name implies this fungus produces an unattractive gray mould over the surface of the fruit. Infection may become established in the fruit prior to harvest and then continues to develop during storage. The fungus produces huge masses of spores which spread the infection from fruit to fruit within the storage area. The potential to use cold storage temperatures to limit loss to Botrytis is limited by the low temperature tolerance of the host fruit. Botrytis is difficult to control with the fungicides presently registered for use after harvest of horticultural commodities.

The potential to use aromatic oils to control *Botrytis* grey mould of strawberries was evaluated using a similar experimental set up as for the seed testing. Strawberries were picked from a U-Pick orchard where no fungicides were applied for disease control. Berries infected with *Botrytis* grey mould were observed in the orchard during fruit picking, at approximately one every 1.5m. The berries were picked in the morning of July 24, were sorted in the afternoon and placed in the treatment pails by 3pm. Five large and five small ripe berries were placed on each of the wire grids in the plastic tubs, the 1:20 strength oils were added as for the seed experiment and the tubs were sealed. One extra treatment was included – a tub with berries on the wire grid, with no oil treatment, was left without a lid on, so development of disease could be monitored (Figure 3.2.2.4). The berries were not cooled after picking, and the containers were kept at room temperature to ensure rapid development of disease.

Results and Discussion:

After three days the strawberries in the open containers were showing fungal growth on the surface (Figure 3.2.2.5). The plastic containers were opened to evaluate the effect of the oil treatments. The fruit in all of the containers were heavily overgrown with a grey fluffy mycelium that appeared to be *Rhizopus* (Figures 3.2.2.6, 7 and 8). There was no evidence that this fungus had been controlled by any of the treatments under these conditions. The berries were in close contact with each other and it appeared that the fungus had spread rapidly over all the berries. It had been anticipated that *Botrytis* would be a storage pathogen for the fruit but *Rhizopus* is a frequent soft rot pathogen of many stored fruit and vegetables.

c) VEGETABLE

Materials and Methods:

Black scurf caused by Rhizoctonia solani is a common disease issue in seed and table potatoes. The presence of the black resting forms (sclerotia) of this fungus on the surface of table potatoes renders them unacceptable in the table market. Seed tubers contaminated with Rhizoctonia are at increased risk of seed piece failure and acceptable

limits of Rhizoctonia infection are set out in CFIA standards for seed potatoes.

Control of Black scurf of potato, caused by *Rhizoctonia solani*, using essential oils was assessed using the same treatment set up as described for strawberry. The concentration of the aromatic oils was increased to 50% as the previously tested concentrations (5%) were not effective in controlling the storage rot of strawberry.

The oil treatments used were:
Anise, caraway, coriander and dill – diluted to 50% aromatic oil in mineral oil
AITC – diluted to 5% in mineral oil
Mineral oil check
Control - no chemical.

Tubers heavily infected with *Rhizoctonia solani* were selected from a Norland seed lot that had been stored in the cooler at 6°C since spring 2009. The infected tubers were cut so that each had an eye (to check for impact of the essential oils on sprouting) and an area of black scurf. One tuber piece was placed on the grid in each tub.

To evaluate the effect of the increased concentration of the essential oils on seed pathogens a sample of chickpea seeds was also placed on the wire mesh in the same containers. The containers were incubated for three weeks at room temperature.

Results and Discussion:

Chickpeas - Heavy surface contamination with a *Penicillium* species developed on the chickpea seeds, presumably because of the presence of this fungus on the surface of the potato tubers. The seeds were sterilized in 50% bleach for 3 minutes and rinsed twice with 10% bleach to try and remove the *Penicillium* contaminants before plating but the seeds all developed fungal infections of *Penicillium*, *Rhizopus*, or *Ascochyta*. No control of the fungal growth was shown by any of the treatments under these conditions.

Potatoes - some of the potato tuber pieces developed bacterial soft rot as a function of the warm moist assay conditions. There were no clear cut differences in the amount of soft rot observed for the various essential oil treatments. There was enough intact skin to allow assessment of the treatment effects on Rhizoctonia. Small areas of potato skin with heavy growth of *Rhizoctonia* were removed from the tubers, and were sterilized in 10% bleach for 30 seconds. Three pieces of skin taken from each tuber were placed in three separate small petri dishes containing PDA. The plates were incubated at room temperature for seven days and were examined for growth of *Rhizoctonia*.

All of the pieces of skin plated out produced colonies of *Rhizoctonia*, *Penicillium* or bacteria. *Rhizoctonia solani,* the causal organism of black scurf, was visible as black fungal mycelium on the surface of the tubers. *Penicillium* is a saprophytic fungus frequently found on the surface of potatoes but is not generally considered to cause any disease problems. There appeared to be a significant effect of the aromatic oils on the survival of *Rhizoctonia* and other organisms on the tubers (Table 3.2.2.2).

Table 3.2.2.2. Effect of aromatic oil treatment for three weeks on the viability of *Rhizoctonia solani* on infected potato tuber skin, after seven days incubation on PDA plates at room temperature .

Aromatic oil treatment	No. of skin pieces (max. of 9) infected with:		
	Rhizoctonia	*Penicillium*	Bacteria
Coriander (50%)	1	1	7
Caraway (50%)	0	0	9
Anise (50%)	3	3	3
Dill (50%)	0	2	7
AITC (5%)	0	0	9
Mineral oil	7	2	0
No oil present	8	1	0

When no oil or mineral oil only was present mainly *Rhizoctonia* grew out of the skin pieces, and no bacterial growth was present. When anise was present the skin pieces were equally infected with *Rhizoctonia*, *Penicillium* and bacteria. In the coriander and dill treatments, bacteria were predominant and in the AITC and caraway treatments only bacterial colonies grew out of the skin pieces. It appeared that the aromatic oil treatments reduced the viability of *Rhizoctonia solani*, particularly the caraway and AITC treatments, but that this allowed development of bacterial colonies. It is possible that these treatments could reduce the presence of fungal pathogens but tubers might then be more susceptible to soft rot.

The impact of the oil treatments on sprouting was not evaluated as the tuber pieces were small and tissue breakdown occurred. The effect on sprouting could be determined more accurately on whole tubers. Carvone has been used to inhibit sprouting and it is likely that the other oils would impact sprout development too.

Conclusions:

The oil treatments, in particular caraway and AITC, appeared to have potential to reduce the viability of *Rhizoctonia* on potato tubers. This would be extremely beneficial for potato growers as not only is black scurf a skin blemish that reduces the marketability of table potatoes but the pathogen also causes significant yield losses when it attacks roots and stolons. However the possibility that soft rot may be increased by an oil treatment needs to be examined as soft rot is a significant problem in potato storages. The conditions used in these experiments were very conducive to soft rot development (no air

circulation, high humidity, room temperatures, wounded potato tissue) and further testing should be conducted under more normal storage conditions. It is also important to take into consideration the effect of oil treatments on sprouting; for seed tubers it is important not to interfere with sprouting but for table potatoes sprout inhibition would be advantageous.

Overall conclusions:

Initial evaluation of the impact of aromatic oil treatments on the growth rate of pure cultures of fungal pathogens in petri dishes indicated that some oils reduced pathogen growth. It appeared that the oils were effective even at relatively low concentrations which would be important in terms of both cost efficiency and reduced potential for adverse side-effects like flavour transfer. However these low concentrations of essential oils did not reduce the levels of seed infection by a number of pathogens. The development of storage rot of strawberries was also not retarded by exposure to realtively dilute concentrations of essential oils. Oil treatments at a higher concentration did appear to reduce the viability of black scurf of potato. This could be an effective product for use in potato storages providing that an increase in soft rot development did not occur, and that sprouting of seed tubers was not affected.

A limited range of test conditions and oil concentrations were used in the experiments reported and future research could concentrate on trying a wider range of concentrations under storage conditions normally used for specific crops. The cost effectiveness of treatments also needs to be considered once an effective concentration under "field" conditions has been determined. Use of aromatic oils as disease deterrents would be advantageous to both spice growers and other crop producers in Saskatchewan and the potential for use of these oils should be evaluated more extensively.

4. PERSONNEL

J.R. Thomson – Research Associate (part-time)
M. McArthur – Research Assistant (part-time)

5. EQUIPMENT

No capital expenditures.

6. PROJECT DEVELOPED MATERIALS

None.

7. PROJECT PHOTOS

Available in separate file.

8. ACKNOWLEDGEMENTS

Support of this research by the Agriculture Development Fund of Saskatchewan Agriculture was acknowledged at all available opportunities.

9. EXPENSE STATEMENT

Research Accounts, University of Saskatchewan will provide a detailed accounting.

Figures in Aromatic Oil report – February 2010.

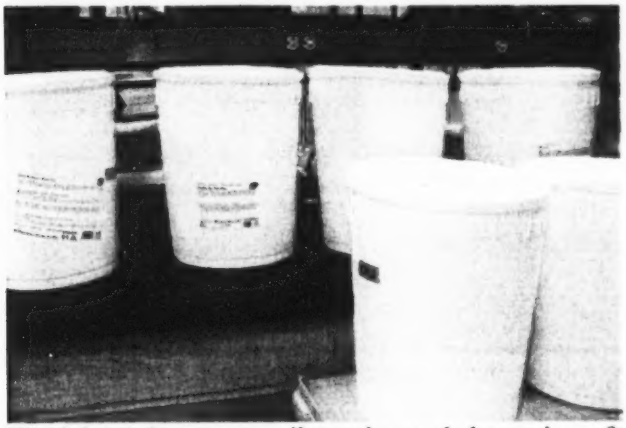

Fig. 3.2.1.1. Ice-cream pails used as sealed containers for exposing inoculated plates, strawberries and potatoes to aromatic oil treatments.

Fig. 3.2.2.1. Seed plated after exposure to aromatic oils treatments for two weeks.

Fig. 3.2.2.2. Barley seed plated for 5 days after aromatic oil treatment.

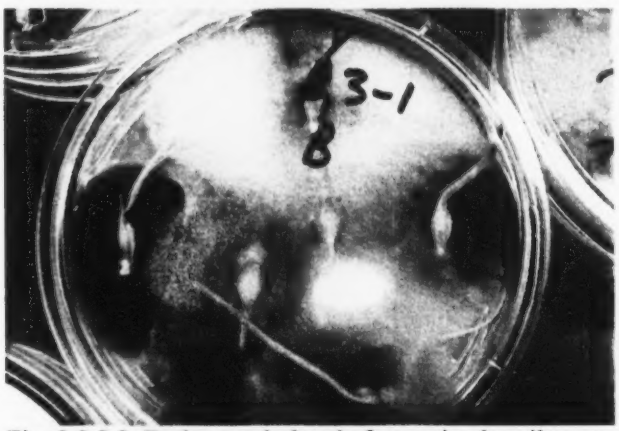

Fig. 3.2.2.3. Barley seed plated after coriander oil treatment, showing fungal growth and germination of seed.

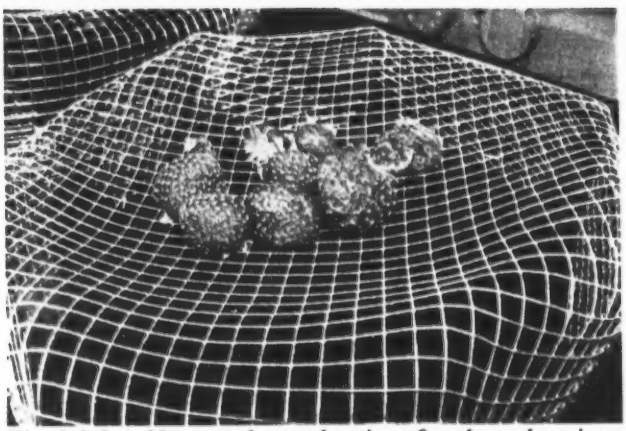

Fig. 3.2.2.4. Untreated strawberries after three days in open air in laboratory.

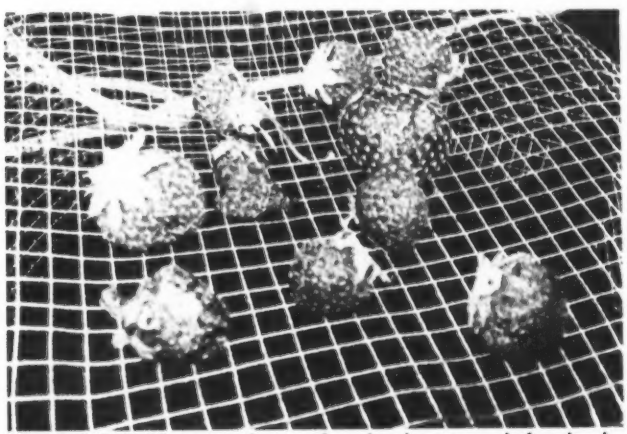

Fig. 3.2.2.5. Untreated strawberries in open air beginning to develop rot after three days.

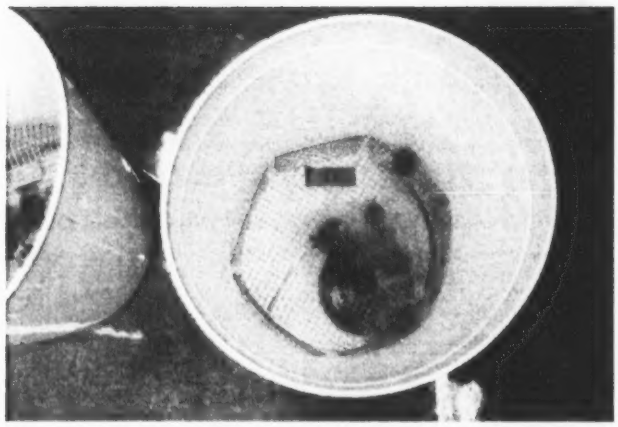

Fig. 3.2.2.6. Fungal growth developed on fruit in containers for three days.

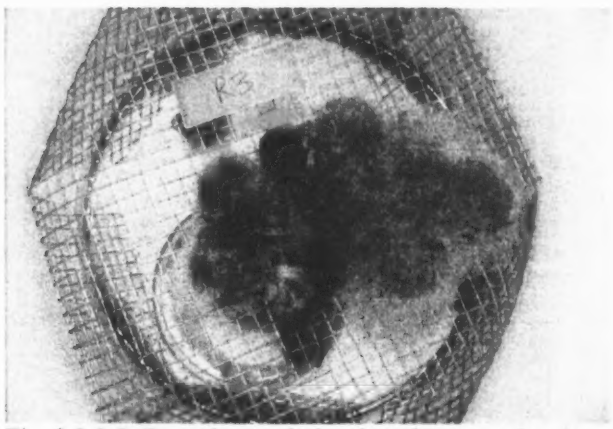

Fig. 3.2.2.7. Fungal growth developed on strawberries treated with dill oil
for three days.

A.

B.

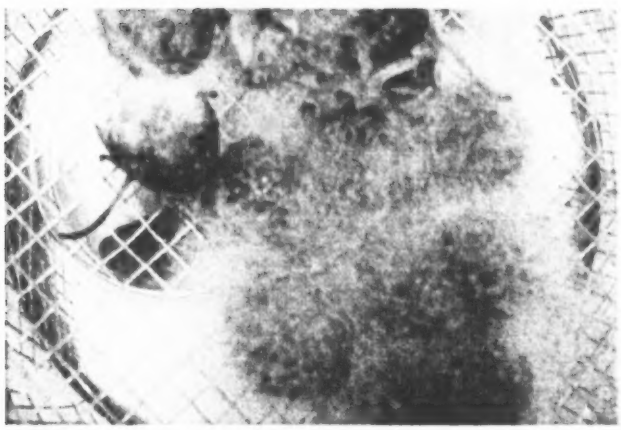

Fig. 3.2.2.8. A & B. *Rhizopus*-like growth on strawberries after three days in container.